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III. Co-transfection with papovavirus and retrovirus DNA using recombinant DNA prepared in vitro.

The introduction of purified pieces of functionally interesting DNA into eukaryotic cells is an important means to deciphering genetic regulation in higher organisms. In many cases, the DNA of interest does not encode functions for which genetic selection procedures are available. We have developed a procedure for circumventing this difficulty by linking transforming regions of a papovavirus genome to pieces of retroviral DNA lacking selectable markers. The approach has the additional virtues of not requiring expression of the retrovirus DNA and of allowing an assessment of signals thought to be important in the integration and expression of the two types of viral genomes.

We joined two fragments of polyoma virus DNA, which together contain an intact early region, and a fragment of transformation-defective avian sarcoma virus (ASV) DNA, which contains the *env* gene of ASV and sequences from the termini of viral RNA. (The ASV DNA was provided by Dr. W. DeLorbe of the University of California, San Francisco, after cloning in λgt WES.) The desired recombinant molecule was isolated as a linear species from other products of ligation of the three fragments by digestion with the restriction endonuclease *BamHI* and preparative gel electrophoresis. After confirming the structure of the recombinant DNA, it was used to transfect rat-1 cells with the calcium phosphate technique. Most of the resulting transformed cells contained from 1 to 3 copies of recombinant DNA. Mapping studies indicated that the recombinant molecules did not circularize during transfection, since the integrated copies were approximately co-linear with the transfecting DNA, though lacking small regions from the termini of the recombinant molecules. Preliminary analysis of the ASV-specific RNA in several transformed lines indicates that few or no stable transcripts are synthesized from this region of the recombinant DNA's (tests performed by S. Ortiz, University of California, San Francisco). The significance of these observations with respect to signals for integration and expression of retroviral DNA is being evaluated by application of these methods to other combinations of molecules and host cells.